BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

19. Japan Patent Agency
11. Patent Application
12. Public Patent Report (A)SHO59-138202

	51. Int. Cl.³	I.D. Number	Intra-Agency Number	43. Opened: August 8, 1984
	C 08 B 37/00		7133-4C	
//	A 61 K 31/73	ACB	6675-4C	

Examination Request-Final Request
Number of Request Items-2 (5 pages)

54. Name of Invention: New sulfated polysaccharide containing a sulfate amino-sugar and its production method

21. Patent Application: SHO58-12044
22. Applied for: January 27, 1983
(for patent law 30 item 1: October 1, 1982, presented in "Polymer Society Manuscript Vol.31, Number 6")

72. Inventor: H. Kuzuhara — 11-27 Kami-yasumatsu, Tokorozawa-shi
72. Inventor: S. Uryu — 3-5-26-519 Aoi, Adachi-ku, Tokyo
72. Inventor: K. Hatanaka — 5-19-17-305 Ogikubo, Suginami-ku, Tokyo
72. Inventor: H. Matsuzaki — 4-10-10 Narita-higashi, Suginami-ku, Tokyo
71. Applicant: Rikagaku Kenkyujo — 2-1 Hirozawa, Wako-shi
74. Representative: Attorney Nakamura, 4 others

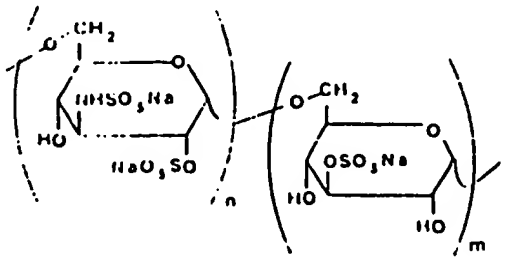
Specifications

1. Name of the invention:

New sulfated polysaccharide containing an amino-sugar and its production method

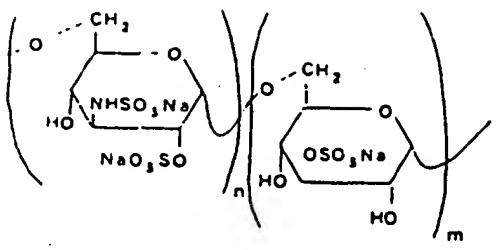
2. Claim

(1) The sulfated polysaccharide containing an amino-sugar is shown in the following equation.



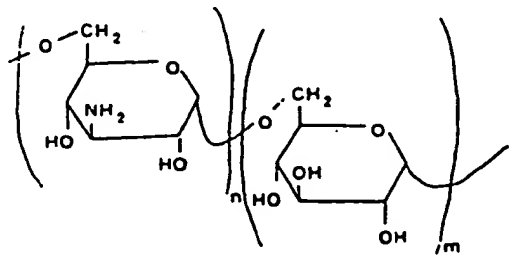
(In the equation, n/m is 1 - 0.2, m+n is an integer from 30 - 150)

(2) The production method of the sulfated polysaccharide containing an amino-sugar shown in equation:



(m/n are same as above)

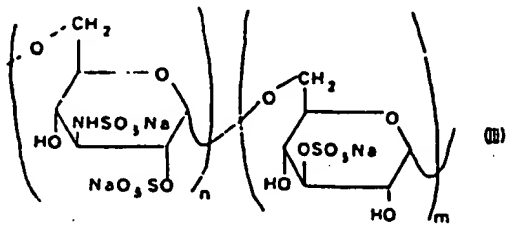
which is characterized by sulfation of the polysaccharide containing an amino-sugar shown in equation:



(In the equation, n/m is 1 - 0.2, m+n is an integer from 30 - 150)

3. Detailed specifications

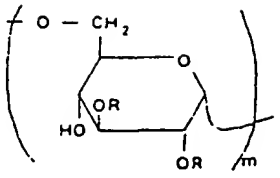
The invention is a new sulfated polysaccharide containing an amino-sugar shown in the following equation and its production method.



(In the equation, n/m is 1 - 0.2, m+n is an integer from 30 - 150)

In the polymer, D-glucopyranose combined with α-1,6 is dextran which is produced by a microorganism such as Leuconostoc mesenteroides. Dextran is useful itself as a blood plasma filler, but its ester sulfate (dextran sulfate) has a lipo-clearing action and decreases the cholesterol and triglyceride in the blood. It also has an anti-hyaluronidase function and is a cellulose solvent, and it is an effective drug for treating high lipemia and arteriosclerosis. Moreover, it is a coagulation inhibitor. These physiological activities are stronger the higher the molecular weight and sulfur content, but at the

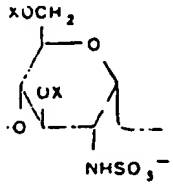
same time, toxicity increases.



R=H : dextran

$R=SO_3Na$: dextran sulfate

On the other hand, heparin, a muco-polysaccharide existing in animal tissue, has a wide range of physiological functions like strong coagulation inhibition and lipo-clearing. These activities are very high compared to man-made heparinoid, but the quality of the standard product is not uniform and since the structure is complex, the isolation process is also complicated. Heparin is characterized by containing sulfated amino-sugar in the molecules, for example the unit below.



(x shows H or SO_3^- in the equation)

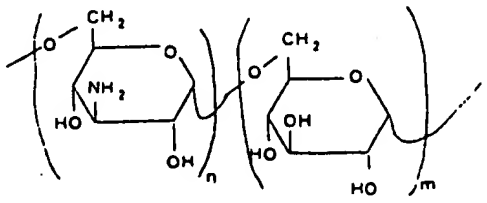
Therefore, if a polysaccharide and its sulfate which contain a sugar chain united with α-1,6 as in dextran, as well as an amino group as in heparin, in the molecule can be synthesized, various physiological functions can be anticipated. However, since this kind of amino polysaccharide does not occur naturally, it must be synthesized, but man-made synthesis of a polysaccharide by the merging of an amino-sugar monosaccharide is technically very difficult. However, first, according to the inventors, by using an azide sugar as the initial material of the amino-sugar, in the beginning, an amino-sugar polymer which has a high degree of polymerization, a sugar chain united with α-1,6, and in the molecule an amino group like in heparin, and its copolymer with D-glucose was synthesized. (see patents 57-180603, 57-180604, 57-180605, and 57-180606)

Furthermore, the inventors diligently researched the synthesis of a sulfate of the

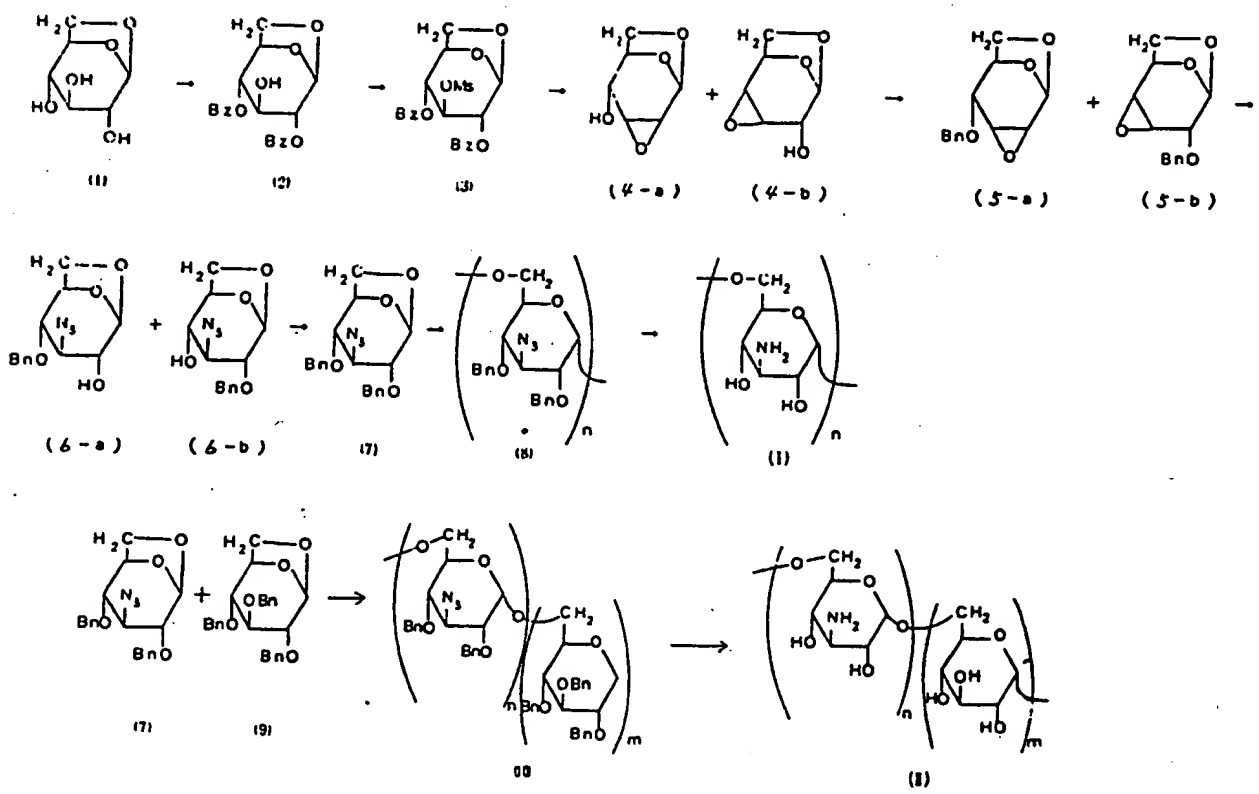
above compound expected to inhibit coagulation and lipo-clear. As a result, this was initially successfully synthesized, resulting in the completion of the invention.

The invention is described below.

The initial material of the invention is shown in the equation:



(n/m is 1 - 0.2, n+m is an integer from 30 - 150)
and the examples of synthesis are as follows.



(In the equation, Bz is benzoyl group, Ms is methanesulfonyl group, Bn and m are the same as in earlier equations.)

Compound (2) is easily attained by treating 1,6-anhydro-β-D-glucopyranose (1) derived from β-D-glucopyranose with benzoylchloride (M. Cerny et al., Collection

Czechoslov, Chem. Commun., Vol. 26, 2542 (1961)).

Compound (2) is dissolved in dry pyridine and frozen. As it is agitated, methanesulfonylchloride is dripped in and gradually rises to room temperature. After 3 hours, when opened in a large amount of ice water and agitated, coarse crystals of compound (3) are extracted. After separation(?), rinsing and drying, it is recrystallized from methanol, producing white crystals of compound (3).

Compound (3) is dissolved in chloroform and frozen. Sodium methoxide solution prepared by dissolving metallic sodium in methanol is dripped into this during agitation. After the mixture sits at room temperature overnight, it is neutralized in 5% hydrochloric acid, and dried by vacuum concentration. The residue is 5 times extracted in acetone, when the extracted solution is vacuum concentrated, an oily material is produced. When this is refined by silica gel chromatography (solvent used chloroform-methanol, 100:1 v/v), a mixture of compounds (4-a) and (4-b) is produced.

The mixture of compounds (4-a) and (4-b) is dissolved in dry tetrahydrofuran, frozen sodium hydride (60% purity) is added and agitated for 30 minutes. Then benzyl bromide is added and reacted at room temperature for four hours. After adding saturated ammonium chloride and agitating for 30 minutes, it is 3 times extracted in ethyl. After the extracted solution is dried in magnesium sulfate anhydride, it is vacuum concentrated; when the oily material produced is refined by silica gel chromatography (solvent used benzene-ethyl acetate, 20:1 v/v), a mixture of oily compounds (5-a) and (5-b) is produced.

The mixture of compounds (5-a) and (5-b) is dissolved in a mixed solvent of ethanol and saturated ammonium chloride water, sodium azide is added and agitated 60 hours, and heat refluxed. After it cools, distilled water is added, the ethanol is vacuum removed, the remaining aqueous solution is extracted in chloroform. After drying the extracted solution in magnesium sulfate anhydride, it is vacuum concentrated, producing an oily material. When it is refined by silica gel chromatography (solvent used benzene-ethyl, 20:1 v/v), a mixture of compounds (6-a) and (6-b) is produced.

The mixture of compounds (6-a) and (6-b) is dissolved in dry tetrahydrofuran and frozen. After sodium hydride (60% purity) is added and agitated for 30 minutes, benzyl

bromide is added. After being agitated for 3 hours at room temperature, frozen saturated ammonium chloride aqueous solution is added and agitated for another 30 minutes. This mixed solution is extracted in ethyl, after the extracted solution is dried in magnesium sulfate anhydride and vacuum concentrated, an oily material is produced. When it is refined by silica gel chromatography (solvent used hexane-ethyl sulfate, 5:1 v/v), oily compound (7) is produced. This is let sit and crystallizes and can be recrystallized from cyclohexane.

On the other hand, compound (9) can be easily produced by treating 1,6-anhydro-β-D-glucopyranose (I) with benzyl bromide.

Copolymerizing and reducing compounds (7) and (9) by the following methods will produce an amino-sugar copolymer (II), the initial material of the invention.

The copolymerization reaction is by dissolving compounds (7) and (9) in a sufficiently dried solvent and reacting them with Lewis acid as a catalyst. The solvent can be methylene chloride or chloroform. The Lewis acid can be phosphorous pentafluoride, antimony pentachloride, or boron trifluoride ethylate, but phosphorous pentafluoride is best with respect to yield and solid regularity of the attained copolymer. The amount of Lewis acid used can be 2 - 7 mole% for the total mole number of compounds (7) and (9), but 2 - 3 mole% is especially suitable. Moreover, by changing the mix ratio of compounds (7) and (9), various copolymers can be produced.

Reaction temperature can be from nearly -30 - -60°C and polymerization time can be from nearly 40 - 60 hours.

It is beneficial to conduct the polymerization reaction in a vacuum or in an inert gas atmosphere (for example N_2 gas).

Thus, in the molecule, a polysaccharide containing an azide sugar (10) with an azide group is produced, and the initial material (II) is produced by then batch reducing this under the same conditions as above.

Thereby, by the following reaction from the initial material produced, the sulfated polysaccharide containing an amino-sugar, the goal of the invention, is produced.

After the above initial material, a polysaccharide containing an amino-sugar, is pretreated and swelled, it is suspended in a suitable solvent. The solvent can be

pyridine, dimethylsulfoxide, or dimethylformaamide. This suspended solution is reacted with a sulfated agent (and the above solvent). The sulfated agent can be chlorosulfon acid, or piperidine-N-sulfuric acid. The amount of the sulfated agent used is 20 - 30 moles for the initial material, amino monosaccharide residue. Reaction time and reaction temperature are different for the solvent and sulfated agent, but 70 - 100°C and 45 - 60 minutes are suitable. After the reaction, it cools and distilled water is added, stopping the reaction. Then it is neutralized by an alkali such as sodium hydroxide, ethanol is added, and the polymer is precipitated. This is centrifugal separated, dissolved in distilled water. After dialysis, by concentration drying, the polysaccharide containing a sulfate amino-sugar, the objective, is produced.

The invention is explained by operation examples below.

Operation Example 1

Polymer (II-a) ($\overline{\overline{DP}}n$=144, n/m=0.96, 100 mg) is dissolved in water (2 ml), ethanol (20 ml) is added, and it is precipitated. After centrifuge separation, and after agitation separation in ethanol and then ethyl, it is suspended in 8 ml previously dried pyridine. This suspended solution and chlorosulfon acid (1 ml, 15 mmol) previously reacted at 0°C are added to 6 ml dry pyridine, soaked in boiling water, and reacted for one hour. After it cools, 20 ml of distilled water is added and the reaction stops. It is neutralized by 2.5 N sodium hydroxide aqueous solution (7.5 ml), ethanol (50 ml) is added, the polymer is sunk. This is centrifugal separated and dissolved in distilled water, after it is dialyzed for 3 days, a sulfated polysaccharide containing an amino-sugar (III-a) is produced by concentration and freeze drying. (168 mg, 76.2%)

[Physical properties of copolymer (III-a)]

	C	H	H	S
Ultimate analysis value : observed value	17.40	3.39	1.55	14.65
Calculated value $C_6H_{8.589}O_{10.211}N_{0.489}S_{1.90}Na_{1.90}\cdot 3H2O$	17.59	3.59	1.68	14.87

(sulfation rate: per sugar-residue SO_3^- 1.90 units)

IR: 580 cm^{-1} (M)
800 cm^{-1} (M)
1240 cm^{-1} (s, broad)
1510 cm^{-1} (W)

$[\alpha]_D^{25}$=+100.2° (C=1.0, H_2O)
$[\eta]$=0.07 (in H_2O, 30°C)

Operation Example 2

Other than using copolymer (II-b) ($\bar{\bar{O}}\bar{\bar{P}}n$=100 - 120, m/n=4.76, NH_2 group 0.114 mmol, OH group 1.74 mmol, 100 mg), if the same reaction and post treatment as in Operation Example 1 are conducted, a sulfated polysaccharide containing an amino-sugar (III-b-1) is produced. (176 mg, 72.0%)

[Physical properties of copolymer (III-b-1)]

	C	H	H	S
Ultimate analysis value : observed value	22.76	4.46	0.67	10.82
Calculated value $C_6H_{9.115}O_{8.025}N_{0.185}S_{1.07}Na_{1.07}$	22.16	4.68	0.80	10.55

(sulfation rate: per sugar-residue SO_3^- 1.07 units)
IR: 580 cm^{-1} (M)
800 cm^{-1} (M)
1240 cm^{-1} (s, broad)
$[\alpha]_D^{25}$=+109.6° (C=1.0, H_2O)
$[\eta]$=0.15 (in H_2O, 30°C)

Operation Example 3

After using 100 mg copolymer (II-b) in swelling treatment under the same conditions as in Operation Example 1, it is dissolved in previously dried dimethyl sulfoxide (28 ml) and made 40°C. Piperidine-N-sulfuric acid (617 mg, 3.73 mmol) is added to this solution and it is reacted for one hour at 80°C while being agitated. After distilled water (70 ml) is added and the reaction stops, it is neutralized by NaOH of 1N. After it is dialyzed for 3 days in distilled water, it is concentrated and freeze dried, producing a sulfated polysaccharide containing an amino-sugar (III-b-2). (200.5 mg, 98%)

[Physical properties of copolymer (III-b-2)]

	C	H	H	S
Ultimate analysis value : observed value	20.36	3.97	0.71	15.06
Calculated value $C_6H_{8.525}O_{9.795}N_{0.185}S_{1.66}Na_{1.66}\cdot 2H2O$	19.62	3.41	0.71	14.49

(sulfation rate: per sugar-residue SO_3^- 1.66 units)
IR: 580 cm^{-1} (M)
610 cm^{-1} (M)
1240 cm^{-1} (s, broad)
Ninhydrin reaction: negative
$[\alpha]_D^{25}$ +102.7° (C=1.0, H_2O)
$[\eta]$=0.07 (in H_2O, 30°C)